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Relationship of Cyclin D1 Expression with Histopathological Grading and Metastasis of Breast Cancer: A Cross-Sectional Study

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ABSTRACTS

Breast cancer (BC) is still a significant health problem in the world, with hormonal therapy as an essential component in its management. Even though it has been stated that it may play a role in the oncogenesis process, the expression profile of Cyclin D1 has not been further analyzed concerning survival and the incidence of breast cancer metastasis. This study investigates the relationship of Cyclin D1 expression with histopathological grading and metastasis in BC.

The research used a cross-sectional study on eligible breast cancer patients in Wahidin Sudirohusodo Hospital and its network hospitals in Makassar. Cyclin D1 expression was examined based on immunohistochemical examination and interpreted using the Allred Scoring method. Histopathological grading is assessed on standard histopathological examination and interpreted based on the Modified Bloom-Richardson Histologic Grading or Nottingham Histologic Score system.

Metastatic status is assessed based on the results of supporting examinations. The Chi-Square test is the primary analysis in this study. Of 57 participants, this study found no association between menopausal status and tumor type and the expression of cyclin D1. In contrast, the luminal subtype of tumor tissue, histopathological grading, and metastasis status were associated with cyclin D1 expression ($p<0.01$).

High expression of Cyclin D1 can be related to the severity of histopathological grading as well as the emergence of metastases to other organs in breast cancer. Future studies with more variables, i.e., Rac1 expression and Paxillin mutase, are required to assess Ccnd1-Cdk4-paxillin-Rac1 and its relationship with BC metastasis.

Keywords: Breast Neoplasms, Carcinogenesis, Cyclin D1, Cross-Sectional Studies

Introduction

According to data from Globocan 2020, the global incidence of cancer has reached approximately 19.3 million new cases annually, with mortality figures attributed to cancer estimated at 10 million deaths per year. Among these, breast cancer (BC) emerges as the predominant malignancy, accounting for 2.3 million cases, which represents 11.7% of all cancer cases across both genders. Precisely, in the female population, BC constitutes 24.5% of all cancer diagnoses, with the same number of cases (2.3 million), underscoring its significant impact on women's health (1).

Over the past decade, clinical research has unveiled innovative therapeutic approaches within clinical practice characterized by integrating targeted therapy alongside hormone therapy. A meta-analysis has demonstrated that the combination of CDK4/6 inhibitors with hormone therapy surpasses conventional hormone therapy alone in enhancing progression-free survival (PFS) when administered as either first or second-line treatment. Furthermore, it has been observed that no chemotherapy regimen, whether accompanied

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by targeted therapy or not, significantly outperforms the combination of CDK4/6 inhibitors with hormone therapy in terms of PFS (2,3).

Cyclin D1, encoded by the CCND1 gene, is recognized as an oncogene. Its overexpression has been frequently observed in BC cases. As one of the key target genes for estrogens, cyclin D1 mediates the mitogenic effects of these hormones. Research in BC has demonstrated a correlation between cyclin D1 expression and positive estrogen receptor (ER) status, indicating an association with well-differentiated, low-grade, and slow-growing subtypes of breast cancer. Additionally, the assessment of cyclin D1 alongside CDK4 expression levels has been identified as a crucial prognostic indicator, offering insights into patient survival rates and metastasis risk in breast cancer (4).

In the study conducted by Siraj et al., involving 1003 BC patients from the Middle East, it was observed that estrogen receptors were positively identified in 656 cases. Among these, a significant proportion, 476 cases (72.6%), exhibited overexpression of Cyclin D1. Similarly, progesterone receptors were positive in 574 cases, with 422 of these cases (73.5%) also showing overexpression of Cyclin D1. The research highlighted a notable prevalence of Cyclin D1 overexpression in luminal subtype breast cancers, particularly within the Luminal A subtype (5). Furthermore, a comprehensive review of the study emphasized that Cyclin D1 accumulation is a crucial marker for cellular proliferation. The findings suggest that the presence of cytoplasmic Cyclin D1 could indicate the tumor's invasive capability. Consequently, the study underscores the importance of assessing Cyclin D1 distribution within tumors. Additionally, the research points to the role of CDK4/6 in facilitating cell migration and invasion processes mediated by Cyclin D1, indicating a dependency of these mechanisms on Cyclin D1 expression (6).

Despite existing studies acknowledging the potential role of Cyclin D1 in the oncogenesis, survival, and metastatic spread of BC, there still needs to be a significant gap in both qualitative and quantitative understanding of Cyclin D1 expression profiles in BC within the Indonesian population. Consequently, this study aims to rigorously assess the expression levels of Cyclin D1 and explore its association with pathological grading and the incidence of metastasis in BCr cases.

Material and Methods

This study employed a cross-sectional methodology to evaluate BC patients at Dr. Wahidin Sudirohusodo Hospital and its affiliated hospitals from July 1st, 2023, to November 30th, 2023. Eligible patient tissue samples were subjected to Hematoxylin and Eosin staining techniques within an accredited laboratory setting. The research protocol received ethical approval from the Health Research Ethics Committee of our institution, under the approval number 823/UN4.6.4.5.31/PP36/2023, dated October 27th, 2023.

The sample size for this study was determined using the formula for cross-sectional study design calculations, taking into account a significance level (α) of 5%, a power (β) of 80%, and the proportions of interest P1 = 0.6 and P2 = 0.25. This resulted in a minimum required sample size of 40. Inclusion criteria for participants were as follows: a confirmed diagnosis of BC based on histopathological examination, age between 30 and 60 years, and classification within the luminal subtype as determined by immunohistochemical analysis. Exclusion criteria were established to omit individuals diagnosed with any cancer types other than BC, individuals suffering from diabetes mellitus, and those who failed to comply with study protocols.

Defintion and Objective Criteria: In this investigation, BC is delineated as a malignancy emanating from the breast tissue's ductal epithelium or lobular regions. The classification system utilized for this study adheres to the 2010 guidelines set forth by the American Joint Committee on Cancer (AJCC), which categorizes tumors based on size (T), the presence or absence of distant metastases (M), and the incidence of regional lymph node metastases (N), facilitating the staging of BC into four primary stages: I, II, III, and IV. Furthermore, the histopathological grading of BC is determinative of the malignancy's prognostic outlook and is indicative of the tumor's aggression potential. This grading was assessed using the Modified Bloom-Richardson Histologic Grading system, also known as the Nottingham Histologic Score system, providing a systematic approach to evaluating the histological characteristics of breast cancer and its potential for progression and response to treatment (7).

Tissue Preparation and Histopathological Examination: Cyclin D1, a protein with a molecular weight of 36 kDa, is encoded by the CCND1 gene on chromosome 11q13. It is ubiquitously expressed in various normal human cells, except those originating from bone marrow stem cell lines. Cyclin D1 expression levels were evaluated through

immunohistochemical staining, and results were quantitatively assessed using the Allred Scoring system. In this context, Allred Score values ranging from 4 to 6 and 7 to 8 are classified as moderate and high, respectively, which are considered indicative of excessive (Positive) Cyclin D1 expression. Conversely, Allred Score values 0 to 1 and 2 to 3 are categorized as negligible and low, respectively, and are interpreted as not indicative of excessive (Negative) Cyclin D1 expression (8).

Metastatic status is obtained from the spread of breast cancer cells to organs other than the breast. Determination of metastases is based on the results of supporting examinations, namely chest radiograph, abdominal ultrasound, and radiograph of associated skeletal symptom.

Statistical Analysis: The statistical analyses will be performed using SPSS software, version 20.0 (IBM Corp., Armonk, NY, USA), tailored to the data's measurement scale and the research objectives. The data will be presented as frequencies and percentages. The association between cyclin D1 expression and the histopathological grading and metastasis in luminal breast cancer will be evaluated using the Chi-square test. The level of statistical significance will be set at a p-value of less than 0.05.

Results

Fifty seven people with BC participated in this study. Table 1 provides a summary of the participants' clinical and demographic data. Based on menstrual status, premenopausal women are slightly more likely than postmenopausal women to experience BC. The results of histological examination showed that the majority of samples in this study suffered from invasive ductal breast carcinoma, luminal type B, and were moderately differentiated in their histopathological grading. In addition, the majority of samples did not experience metastasis.

The analysis of the association between Cyclin D1 expression wiith menstrual status, histopathological examination results, and metastasis state can be seen in Table 2. Menopausal status and tumor type were not associated with the expression of cyclin D1. In contrast, the luminal type of tumor tissue, histopathological grading, and metastasis status were associated to cyclin D1 expression ($p<0.01$). Most samples had excessive expression of cyclin D1 in luminal B followed by high expression of Ki67. Apart from that, most samples also had excessive expression of cyclin D1 on histopathological grading which was poorly differentiated and had metastases.

Discussion

In this research, it was observed that a majority of the BC patients were premenopausal. According to the study conducted by Mohapatra and Sharma, it was found that 52.7% of breast carcinoma cases occurred in premenopausal women, while 45.3% were reported in postmenopausal women (9). This contrasts with the findings of Surakasula et al., where the prevalence of breast cancer was higher in postmenopausal women, accounting for 52.0% of the cases. These discrepancies highlight the complexity of breast cancer etiology and suggest that multiple factors, including hormonal changes associated with menopause, may contribute to the development of the disease (10).

The current study observed no statistically significant correlation between menstrual status and the expression levels of Cyclin D1. This finding is consistent with the results presented in the research conducted by Parvin et al., which also reported a lack of significant association between menopausal status and the intensity of Cyclin D1 expression (11).

In the present study, the predominant subtype of breast carcinoma identified was invasive ductal carcinoma (IDC). This aligns with the findings of Zangouri et al., who also reported the highest incidence rates of IDC in the breast (12). Further detailed analysis conducted by Mohapatra and Sarma revealed that non-special type IDC constituted 93.7% of the cases, whereas invasive mucinous carcinoma and medullary carcinoma each accounted for 1.0% of the cases (9).

This study found no significant association between tumor type and Cyclin D1 expression levels across a diverse set of malignancies. However, a nuanced analysis reveals a notable trend of Cyclin D1 overexpression in cases of invasive ductal carcinoma of the breast. In a subset study conducted by Parvin et al. involving 50 BC patients, 42 were diagnosed with invasive ductal carcinoma. Within this subgroup, 10 cases (approximately 25%) demonstrated significant overexpression of Cyclin D1. Despite these findings, the study concluded that there is no statistically significant correlation between the histological type of the tumor and the degree of Cyclin D1 overexpression (11).

The study demonstrated a significant predominance of high Ki-67 (Luminal B) expression. Recent investigations by Ritu and Rashmi in India have elucidated a strong correlation between Cyclin D1 and Ki67 markers. Specifically, cyclin D1 is instrumental in cell cycle progression from the G1 to the S phase, underscoring its significance in cellular

Table 1: Clinical and Histopathological Characteristics of Study Participants

Characteristics	n (%)
Menstrual Status	
Premenopause	29 (50.9)
Postmenopause	28 (49.1)
Tumor Histopathological Type	
Premenopause	47 (80.7)
Postmenopause	10 (19.3)
Luminal Subtype	
Luminal A (Ki67 Low)	22 (38.6)
Luminal B (Ki67 High)	35 (74.4)
Histopathological Grading	
Well Differentiated	13 (22.8)
Moderate Differentiated	26 (45.6)
Poorly Differentiated	18 (31.6)
Metastasis Status	
Yes	27 (47.0)
No	30 (53.0)

Table 2: Association Analyisis of Clinical Status, Histopathological Examination, and Metastasis Status With Cyclin D1 Expression

Characteristics	Cyclin D1 Expression		p-value
	High n (%)	Low n (%)	
Menstrual Status			
Premenopause	19 (65.5)	10 (34.5)	0.471
Postmenopause	22 (78.6)	6 (21.4)	
Tumor Histopathological Type			
Premenopause	33 (70.0)	14 (30.0)	0.532
Postmenopause	8 (80.0)	2 (20.0)	
Luminal Subtype			
Luminal A (Ki67 Low)	12 (54.5)	10 (45.5)	<0.01
Luminal B (Ki67 High)	29 (82.8)	6 (17.2)	
Histopathological Grading			
Well Differentiated	2 (15.4)	11 (84.6)	<0.01
Moderate Differentiated	22 (84.6)	4 (15.4)	
Poorly Differentiated	17 (94.4)	1 (5.6)	
Metastasis Status			
Yes	25 (92.6)	2 (7.4)	<0.01
No	16 (53.3)	14 (46.7)	

proliferation mechanisms (13). Furthermore, Ortiz et al. have identified a correlation between Cyclin D1 expression and the expression of estrogen receptor (ER) and progesterone receptor (PR) and an association with the Luminal subtype of breast cancer. These findings reinforce the critical role of cyclin D1 in the pathogenesis of estrogen-induced BC. The mechanism underlying this involvement is primarily through the transcriptional activation of cyclin D1 and c-Myc (14).

The amplification of CCND1 is posited to play a critical role in the initiation and progression of tumors through activating proto-oncogenes. In this investigation, CCND1 amplification was observed in

18% of the cases. A notable correlation was identified between the expression of Cyclin D1 and elevated levels of the Ki-67 protein, as well as with the Luminal B subtype, which indicates a poorer prognosis. In contrast, a study by Mohammadizadeh et al. demonstrated a pronounced association between Cyclin D1 overexpression and the Luminal A subtype, with all Luminal A tumors exhibiting positive overexpression of Cyclin D1. Meanwhile, 80% of Luminal B tumors were positive for Cyclin D1 overexpression, starkly contrasting to merely 40% of triple-negative tumors displaying Cyclin D1 positivity (15).

This study's histopathological grading of tumors predominantly revealed moderately differentiated tumors, with poorly differentiated and well-differentiated tumors following in frequency. This distribution aligns with the findings of a retrospective study conducted by Oluogun et al., which reported a predominance of moderately differentiated tumors, constituting 71% of their observed cases (16).

The present study elucidates a notable correlation between histopathological grading and Cyclin D1 expression, aligning with the findings of Parvin et al., who reported a statistically significant association between Cyclin D1 expression and histological grade ($p < 0.001$).(11) Additionally, the research conducted by Chung et al. highlighted an overexpression of Cyclin D1 in tumors with lower histological grades (17). This correlation implies that Cyclin D1 expression may indicate less aggressive tumor phenotypes. Similarly, Ahlin et al. confirmed a significant relationship between tumor class and Cyclin D1 expression in their investigation (18).

This study delineates a correlation between metastasis in BC and the expression of Cyclin D1, presenting findings that diverge from previous research's findings. In contrast to the study by Ravikumar and Ananthamurthy, which analyzed 39 BC cases and reported no significant association between metastasis and Cyclin D1 expression, the present investigation draws upon a larger cohort (19). Langsenlehner et al., examining 302 breast cancer cases characterized by an overexpression of Cyclin D1, identified that 82.8% of these cases progressed to develop metastases, whereas only 17.2% did not ($p=0.010$). This significant correlation underpins the conclusion that Cyclin D1 expression is indeed associated with an increased incidence of metastasis in breast cancer. Furthermore, the study elucidates the mechanistic pathway by which Cyclin D1 may facilitate tumor cell invasion. The Cyclin D1-Cdk4 complex is proposed to phosphorylate a specific fraction of paxillin associated with cell membranes. This phosphorylation event is pivotal for activating Rac1, which, in turn, triggers the formation of membrane ruffles and cell invasion (20).

The primary limitation of this study stems from the need for a comprehensive multivariate analysis. Considering the multifaceted nature of BC progression, including the roles of hormone receptor status and HER-2 expression, it is imperative to incorporate these variables into the analysis. A joint examination incorporating cyclin D1 expression alongside these factors would yield a more robust understanding of its correlation with histopathological grading and metastasis status.

In conclusion, our comprehensive investigation has elucidated that elevated Cyclin D1 expression correlates with higher histopathological grades and the emergence of metastases in distant organs. This finding underscores the potential role of Cyclin D1 as a prognostic marker for disease progression. However, to comprehensively delineate the mechanistic pathways involving Cyclin D1, further studies incorporating additional variables, such as Rac1 expression and Paxillin mutations, are imperative.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49. (In English).
2. Giuliano M, Schettini F, Rognoni C, Milani M, Jerusalem G, Bachelot T, et al. Endocrine treatment versus chemotherapy in postmenopausal women with hormone receptor-positive, HER2-negative, metastatic breast cancer: a systematic review and network meta-analysis. Lancet Oncol. 2019;20(10):1360–9. (In English).
3. Piezzo M, Chiodini P, Riemma M, Cocco S, Caputo R, Cianniello D, et al. Progression-Free Survival and Overall Survival of CDK

4/6 Inhibitors Plus Endocrine Therapy in Metastatic Breast Cancer: A Systematic Review and Meta-Analysis. Int J Mol Sci. 2020;21(17):6400. (In English).

4. Yang C, Chen L, Li C, Lynch MC, Brisken C, Schmidt EV. Cyclin D1 enhances the response to estrogen and progesterone by regulating progesterone receptor expression. Mol Cell Biol. 2010;30(12):3111–25. (In English).
5. Siraj AK, Parvathareddy SK, Annaiyappanaidu P, Ahmed SO, Siraj N, Tulbah A, et al. High Expression of Cyclin D1 is an Independent Marker for Favorable Prognosis in Middle Eastern Breast Cancer. OncoTargets Ther. 2021;14:3309–18. (In English).
6. Tchakarska G, Sola B. The double dealing of cyclin D1. Cell Cycle. 2019;19(2):163–78.
7. Meyer JS, Alvarez C, Milikowski C, Olson N, Russo I, Russo J, et al. Breast carcinoma malignancy grading by Bloom-Richardson system vs proliferation index: reproducibility of grade and advantages of proliferation index. Mod Pathol Off J U S Can Acad Pathol Inc. 2005;18(8):1067–78. (In English).
8. Gru AA, Allred DC. Molecular Pathology of Breast Cancer. In: Cheng L, Eble JN, editors. Molecular Surgical Pathology. New York, NY: Springer New York. 2013;p. 95–128. (In English).
9. Mohapatra M, Sarma Y. A study on clinico-pathological assessment of response to neoadjuvant chemotherapy in breast carcinoma. J Cancer Res Ther. 2020;0(0):0. (In English).
10. Surakasula A, Nagarjunapu GC, Raghavaiah KV. A comparative study of pre- and post-menopausal breast cancer: Risk factors, presentation, characteristics and management. J Res Pharm Pract. 2014;3(1):12–8. (In English).
11. Parvin T, Das C, Choudhury M, Chattopadhyay BK, Mukhopadhyay M. Prognostic Utility of Cyclin D1 in Invasive Breast Carcinoma. Indian J Surg Oncol. 2019;10(1):167–73. (In English).
12. Zangouri V, Akrami M, Tahmasebi S, Talei A, Ghaeini Hesarooeih A. Medullary Breast Carcinoma and Invasive Ductal Carcinoma: A Review Study. Iran J Med Sci. 2018;43(4):365–71. (In English).
13. Ritu M, Rashmi Y. Carcinoma Breast: Correlation of Immunohistohemical Expression of Cyclin D1 and Its Correlation with Clinicopathological Parameters in Indian Patients at Tertiary Care Hospital. Int J Oncol Res. 2022;5(2). (In English).
14. Ortiz AB, Garcia D, Vicente Y, Palka M, Bellas C, Martin P. Prognostic significance of cyclin D1 protein expression and gene amplification in invasive breast carcinoma. PLoS ONE. 2017;12(11):e0188068. (In English).
15. Mohammadizadeh F, Hani M, Ranaee M, Bagheri M. Role of cyclin D1 in breast carcinoma. J Res Med Sci Off J Isfahan Univ Med Sci. 2013;18(12):1021–5. (In English).
16. Oluogun WA, Adedokun KA, Oyenike MA, Adeyeba OA. Histological classification, grading, staging, and prognostic indexing of female breast cancer in an African population: A 10-year retrospective study. Int J Health Sci. 2019;13(4):3–9. (In English).
17. Chung J, Noh H, Park KH, Choi E, Han A. Longer Survival in Patients with Breast Cancer with Cyclin D1 Over-Expression after Tumor Recurrence: Longer, but Occupied with Disease. J Breast Cancer. 2014;17(1):47–53. (In English).
18. Ahlin C, Lundgren C, Embretsén-Varro E, Jirström K, Blomqvist C, Fjällskog ML. High expression of cyclin D1 is associated to high proliferation rate and increased risk of mortality in women with ER-positive but not in ER-negative breast cancers. Breast Cancer Res Treat. 2017;164(3):667–78. (In English).
19. Ravikumar G, Ananthamurthy A. Cyclin D1 expression in ductal carcinoma of the breast and its correlation with other prognostic parameters. J Cancer Res Ther. 2014;10(3):671. (In English).
20. Langsenlehner U, Hofmann G, Samonigg H, Krippl P, Renner W, Clar H. Cyclin D1 genotype and breast cancer metastasis. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2005;14(7):1844–5. (In English).